Quantitative mast cell analysis and hormone receptor study (ER, PR and HER2/neu) in invasive carcinoma of breast

Rama D. Pyla, R. M. Potekar, Vijayalaxmi S. Patil, Anil K. Reddy, K. V. Sathyashree

Department of Pathology, Shri B M Patil Medical College, Hospital and Research Center, BLDE Deemed to be University, Sholapur Road, Vijayapura, Karnataka, India

Address for correspondence:
Dr. R. M. Potekar, Department of Pathology, Shri B M Patil Medical College, Hospital and Research Center, BLDE Deemed to be University, Sholapur Road, Vijayapura - 586 103, Karnataka, India. E-mail: ratnakar.potekar@bldeuniversity.ac.in

ABSTRACT

Context: Breast cancer constitutes nearly one third of cancers among women. Immune responses caused by neoplastic cells lead to the accumulation of inflammatory cells like mast cells (MCs), macrophages, lymphocytes, and plasma cells around the tumor tissue forming the tumor microenvironment. **Aim:** The study aims at quantifying the role of MCs in different grades of invasive carcinoma of breast with respect to estrogen receptor (ER), progesterone receptor (PR), and Human Epidermal growth factor Receptor 2 (HER2/neu). **Materials and Methods:** This study included 60 cases of invasive carcinoma of breast. Toluidine blue staining was used for quantitative MC analysis and correlated with immunohistochemistry analysis for hormonal markers' positivity—ER, PR and HER2/neu. **Results:** The mean age was 52 years (range: 25–75 years). The average number of MCs in Grade I, II, and III were 24.05, 18.4, and 7.9, respectively, with a significant *P* value. ER, PR, and HER2/neu positivity was found in 60%, 55%, and 32% of the cases, respectively. ER positivity with mean MC count of 23.55 was found in 36 cases, and 33 cases were positive for PR with a mean MC count of 24.18 and a significant *P* value. HER2 positive cases were 28 with a mean MC count of 20.82. **Conclusion:** The presence of MCs in breast cancer is inversely proportional to the grade of tumor, i.e., a maximum number of MCs were seen in low grade tumors. In addition, there is a positive correlation between ER and PR receptor positivity with the presence of MCs in the stroma of breast cancer.

KEY WORDS: Breast cancer, invasive ductal carcinoma, mast cell, toluidine blue, tumor microenvironment

INTRODUCTION

Among women, breast cancer is the most prevalent constituting one third of cancers, followed by lung cancer with respect to mortality. Invasive carcinoma of no special type (NST) with a frequency of about 83% is the most prevalent of all histological types of breast carcinoma.[1]

In recent times, many studies were undertaken with a focus on the cellular and extracellular matrix components present in the tumor microenvironment which constitutes various innate and adaptive immune cells.[2] Immune response caused by neoplastic cells leads to the accumulation of inflammatory cells like mast cells (MCs), macrophages, lymphocytes, and plasma cells around the tumor tissue.[3] Tissue microenvironment changes during tumor formation and during the progression from normal mammary gland to ductal carcinoma *in situ* and finally to invasive ductal carcinoma.

The presence of MCs in tumor tissue was first reported by Ehrlich as early as 1878.[4] MCs are derived from the multipotent hematopoietic bone marrow progenitor cells. While still immature, MCs migrate from the vascular to the peripheral tissue where they mature and are widely distributed throughout the body.[3,5]

MCs, a part of the innate immune system, are recruited and activated in the microenvironment of a developing tumor.[6] The accumulation of MCs helps

in the growth of the tumor as it facilitates tumor angiogenesis by releasing heparin-like molecules. These molecules secrete growth factors such as platelet-derived, nerve, and vascular endothelial growth factor; stem cell factor histamine; and metalloproteases that contribute to tumor invasiveness.[7,8]

MCs harm tumor cells by secreting several cytokines like interleukin 4 (IL-4), IL-1, and IL-6 and tumor necrosis factor α (TNFα), which induces apoptosis of malignant cells and thus has an inhibitory effect on the tumor growth.[8]

Besides breast cancer, MC infiltrate has been studied in non-small cell lung cancer, basal cell carcinoma, colorectal cancer, and pulmonary adenocarcinoma.[6]

This is a comparative study of quantitative MC analysis and hormone receptor study— estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2/neu)—in invasive carcinoma of breast.

MATERIALS AND METHODS

The study was conducted as a prospective study from 2016 to 2018 at the pathology department. Sixty total/modified radical mastectomy specimens of patients diagnosed with invasive ductal carcinoma were included in the study. Patients on radiotherapy/chemotherapy prior to surgery were excluded.

All total/modified mastectomy specimens were collected in 10% buffered formalin. The gross characteristics and specimen borders were noted and cut at an interval of 1 cm and kept for overnight fixation. Further, sections of 4–5 μm thickness were prepared and stained with hematoxylin and eosin (H and E). All sections were examined and reviewed by two pathologists. Grading was done according to the modified Scarff–Bloom–Richardson (SBR) grading system as mentioned in Table 1.

For the demonstration of MCs representative, 3–4 μm thick tissue sections were prepared from formalin-fixed, paraffin-embedded tissues and stained with 0.1% toluidine blue. MC count was done in 400× magnification and in 10 high power fields (HPFs). Average per HPF was noted. The counting of MCs was done in Labomed L × 300i with a field diameter of 0.45 mm.

Three additional 3–4 μm thick sections were taken on the charged slides from the tumor tissue for immunohistochemistry (IHC) and were stained with DAKO® reagents of ER (α Clone EP1), PR (Clone PgR 636), and HER2 (c-erbB-2). ER and PR scoring was done according to Allred scoring system detailed in Table 2. HER2 scoring was done according to the guidelines given from the American Society of Clinical Oncology (ASCO) shown in Table 3.

Statistical analysis

Data was analyzed using the Statistical Package for Social Sciences (SPSS® version 17.0) and Microsoft® Excel 2016. Quantitative data was presented as mean +/- standard deviation, and qualitative data was represented as frequency and percentage (%). Analysis of variance (ANOVA) test was used to describe the association between MC density and histological grade.

A *P*-value of <0.005 was considered to be statistically significant.

RESULTS

The study covered 60 cases with a mean age of 52 years (range: 25–75 years). The invasive ductal carcinoma cases were

Table 1: Semiquantitative method for assessing histological grade in breast. From Elston-Ellis modification of SBR scoring system[9]

Features	Score	
1. Tubule and gland formation		
Majority of tumor (>75%)	1	
Moderate degree (10-75%)	2	
Little or none (<10%)	3	
2. Nuclear pleomorphism		
Small, regular uniform cells	1	
Moderate increase in size and variability	2	
Marked variation	3	
3. Mitotic counts		
Dependent on microscope field area	1-3	
Mitotic count/10 HPFs		
1 point	0-9	
2 point	10-19	
3 point	>20	
Final grading		
Grade I	Total score: 3-5	Well differentiated
Grade II	Total score: 6 or 7	Moderately differentiated
Grade III	Total score: 8 or 9	Poorly differentiated

HPFs: High power fields; SBR: Scarff-Bloom-Richardson

Table 2: Allred system of scoring for ER and PR

Proportion Score	
Score	Percentage of stained cells
0	No cells are ER positive
1	≤1% cells are ER positive
2	1-10% cells are ER positive
3	11-33% cells are ER positive
4	34-66% cells are ER positive
5	67-100% cells are ER positive
Intensity Score	
Score	Intensity of staining
0	Negative
1	Weak
2	Intermediate
3	Strong
Allred Score (Allred score=Proportion Score + Intensity Score)	
Allred score	Effect of hormone therapy
0-1	No effect
2-3	Small (20%) chance of benefit
4-6	Moderate (50%) chance of benefit
7-8	Good (75%) chance of benefit

ER: Estrogen receptor; PR: Progesterone receptor

of Grade II (45% [*n* = 27]), followed by Grade I (33.3% [*n* = 20]) and Grade III (21.7% [*n* = 13]) under the modified SBR grading system. The mean MC count obtained was tabulated, and the cases falling under Grade I had the highest number of MCs with a mean value of 24.05 in 10 HPFs. The mean values of MCs for Grades II and III were 18.4 and 7.9, respectively. Using Kruskal–Wallis test, a statistically significant *P* value of 0.0001 was obtained. The detailed analysis is presented in Table 4. Photomicrograph showing peritumoral MC in case of Grade I is shown in Figure 1.

On IHC, ER and PR positivity were found in 60% and 55% of the cases, respectively. HER2/neu positivity was noted in 46.7% of the cases. A detailed tabulation of the analysis is shown in Table 5. The IHC staining of tumor tissue is shown in Figure 2; Figure 2a shows membrane positivity of HER2 with a score of 3, and Figure 2b and 2c show nuclear positivity. Allred score of 8 was noted with ER and PR respectively.

Table 3: Immunohistochemistry scoring method for HER2

Score to Report	HER2 Overexpression	Assessment of Protein Staining Pattern
0	Negative	No staining is observed, or membrane staining in fewer than 10% of tumor cells.
1+	Negative	A faint or barely perceptible membrane staining is detected in more than 10% of tumor cells. The cells are only stained in part of the membrane
2+	Borderline	A weak to moderate complete membrane staining is observed in more than 30% of tumor cells.
3+	Positive	A strong complete membrane staining is observed in more than 30% of the tumor cells.

HER2: Human epidermal growth factor receptor 2

Table 4 : Average count of MCs in IDC samples

Modified SBR grading	Mean (median) ± of MCs	SD	Kruskal-Wallis test
Grade I (*n*=20)	24.05 (20)	13.92	*P*=0.0001*
Grade II (*n*=27)	18.44 (11)	18.339	
Grade III (*n*=13)	7.923 (7)	9.15	

Note: *Indicates significant difference, MCs: Mast cells; SBR: Scarff-Bloom-Richardson; SD: Standard deviation, IDC: Invasive ductal carcinoma
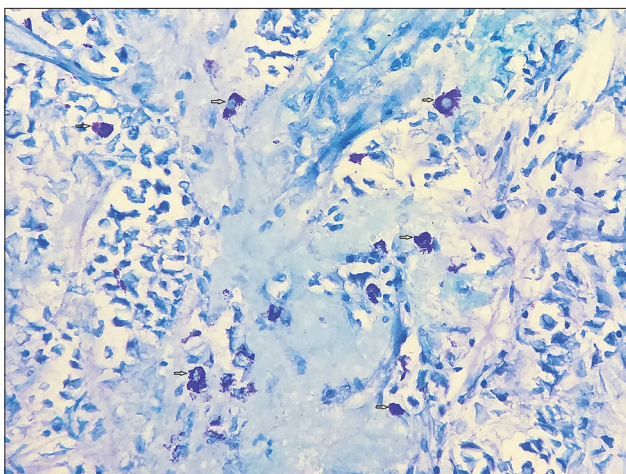

Figure 1: Microphotograph showing MCs (arrow marks) in peritumoral area of Grade I with a mean MC count of 58 (toluidine blue stain, 400×)

MCs were present in all cases of Grade I invasive ductal carcinoma and absent in three cases (11%) of Grade II and two cases (15%) of Grade III.

Out of 36 ER positive cases, 35 (97%) showed a presence of MCs, and in 1 (3%) case MCs were absent. Out of 24 ER negative cases, 20 (83%) showed a presence of MCs, and 4 cases (17%) were without MCs. However, *P* value was found to be insignificant. Out of 33 PR positive cases, 32 (97%) showed a presence of MCs, and in 1 case (03%) MCs were absent. Out of 27 PR negative cases, 23 (85%) showed a presence of MCs, and 4 cases (15%) were without MCs. However, *P* value was not significant. Out of 28 cases with positive HER2/neu receptor, 27 (96%) showed a presence of MCs, and in 1 case (4%) MCs were absent. There were 32 negative HER2/neu receptor cases, in which 28 (87%) showed a presence of MCs and 4 (13%) were without MCs. However, *P* value was not significant. The correlation between ER, PR, HER2/neu and MCs in breast cancer is given in Table 6.

The mean MCs were correlated with the ER, PR, and HER2/neu status. Out of 36 cases with ER positivity, mean of MCs was 23.55 with a *P* value of <0.0001 indicating statistical significance. Out of 33 cases positive for PR, mean of MCs was 24.18 with a *P* value of 0.0019 indicating statistical significance. For 28 HER2/neu positive cases, mean MC count of 20.82 was not statistically significant.

DISCUSSION

Breast cancer is one of the most common cancer among females. With the advancing investigative modalities in breast cancer, the management is evolving which requires precise grading and accurate pathological diagnosis aided by various molecular
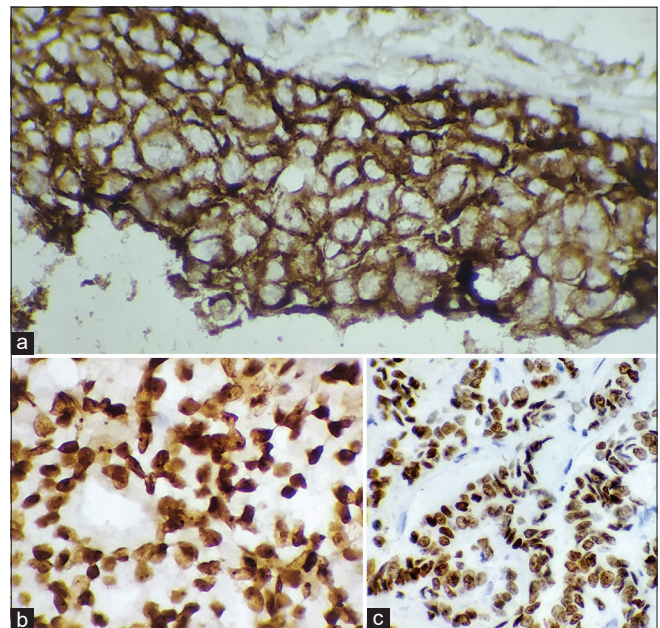

Figure 2: Microphotograph showing IHC staining of tumor tissue with HER2, ER, and PR (a-c)

Table 5: Distribution of cases according to ER, PR, and HER2/neu

	No. of cases	Percentage
ER		
Negative	24	40
Positive	36	60
PR		
Negative	27	45
Positive	33	55
HER2/neu		
Negative	32	53.3
Positive	28	46.7

ER: Estrogen receptor; PR: Progesterone receptor; HER2: Human epidermal growth factor receptor 2

Table 6: Correlation between ER, PR, HER2/neu and MCs in breast cancer

Variables	Mean (Median) ± SD of MCs		Mann-Whitney U test
	Positive	Negative	
ER	23.55 (18) ± 17.02	9.75 (8.0) ± 10.64	U=172.54 P<0.0001*
PR	24.18 (18) ± 18.09	10.52 (10) ± 9.292	U=235.50 P=0.0019*
HER2/neu	20.82 (12) ± 17.99	15.59 (11.5) ± 14.301	U=378.00 P=0.3030 NS

Note: *Indicates significant difference. ER: Estrogen receptor; PR: Progesterone receptor; HER2: Human epidermal growth factor receptor 2; MCs: Mast cells; NS: No significant difference; SD: Standard deviation

techniques. A better understanding of the factors that influence tumor behavior and disease course is gaining importance in today's practice.[10]

Thus, paving way for several new prognostic markers which are being identified for breast cancer. In the current times, this has become a prerequisite as the treatment guidelines recommend adjuvant therapy for the management of many subtypes of breast cancer.[8]

Many researchers are actively investigating the relationship between the tumor and role of stromal inflammatory cells like MCs, fibroblasts, macrophages, lymphocytes, and T-cell subtypes in the initiation and progression of cancer. Tissue microenvironment changes during tumor formation and also during the progression from normal mammary gland to ductal carcinoma *in situ* and lastly to invasive ductal carcinoma. However, very few studies have been done on invasive breast cancer for the demonstration of stromal MCs. The present study discerns the relationship of MCs and invasive carcinoma of breast and the correlation of stromal MCs with positivity of ER, PR, and HER2/neu.[3,6]

Triple negative breast cancers are more aggressive with poor prognosis, occur at an early age, and are not responsive to conventional targeted therapies. MCs have no significant role in triple negative breast cancers.[11]

Among the 60 cases diagnosed as invasive ductal carcinoma on histomorphological examination, the age range was 55–64 years with a mean age of 52 years; Fakhrjou *et al.*[1] and Divyarani

et al.[3] noted similar cases found between 40–50 years with a mean age of 52 years.

All invasive ductal carcinoma cases were graded using the modified SBR grading system into Grade I, II, and III, with the maximum number of cases falling under Grade II followed by Grade I and III. Similar findings were observed by Divyarani *et al.*[3] However, in Heidarpour *et al.*,[8] Grade I cases were a majority, and Glajcar *et al.*[12] noted that Grade III cases were more. Fakhrjou *et al.*[1] has taken equal number of cases in all the three grades.

MCs appear as mononuclear round-to-oval cells with granular cytoplasm. MCs contain granules that can be stained by metachromatic stains, such as toluidine blue and Giemsa stain, and appear purplish pink in color. MCs synthesize factors such as IL-8; heparin and vascular endothelial growth factor which promotes neovascularization and suppresses immune response by histamine; and proteases that helps in metastasis. MCs inhibit tumor growth by synthesizing endogenous peroxidase, a cytotoxic substance, and releasing cytokines like IL-4, IL-6, IL-1, and TNF α. Tryptase synthesized by MCs promotes fibroblast recruitment leading to tumor fibrosis that limits tumor growth and metastasis.[6]

In the present study, MCs stained with toluidine blue were identified by the metachromatic reaction, which was also followed in the studies done by Fakhrjou *et al.*[1] and Divyarani *et al.*[3] Rovere *et al.*,[4] Amini *et al.*,[13] and Glajcar *et al.*[12] used Giemsa, Alcian blue, and tryptase to demonstrate MCs. C-kit (CD117), tryptase, and chymase were also used in some studies. Tryptase positive MCs were negatively associated with tumor size. Tryptase and chymase positive MCs showed an inverse correlation with ki67 expression—this hypothesis demonstrates a protective role of MCs against cancer.[12]

The relationship between MC count and grades of disease was investigated. MCs were mainly found in tumor stroma adjacent to tumor cells and also seen infiltrated within the islands of neoplastic cells. In the present study, MCs were counted in 400□ magnification in 10 HPFs, and the mean MC count was highest in Grade I, followed by Grade II and Grade III. These results correlated with the studies done by Divyarani *et al.*,[3] Jana S *et al.*,[14] and Sang J *et al.*[15] In addition, a study conducted by Heidarpour *et al.*[8] showed that the presence of stromal MCs was correlated with the low grade of the tumor.

Maximum number of MCs were seen in low grade and a lesser number of MCs were seen as the grade of the tumor increased, suggesting that MCs may have an inhibitory role in the development of breast cancer. However, studies conducted by Fakhrjou *et al.*[1] and Kwon *et al.*[10] showed that a higher number of MCs were seen in Grade III as an increase in the number of MCs recruited by the tumor cells contributes to angiogenesis that may facilitate in the expansion of primary tumor leading to increase in its proliferative rate.[10]

It was recently reported that increased stromal MCs identified with immunocytochemistry for c-kit in invasive human breast cancer may be a favorable prognostic sign. However, it has not been used as an independent factor in diagnosis and therapeutic acts yet.

In the present study, ER positive cases were 36 (60%) and negative were 24 (40%). PR positive cases were 33 (55%) and negative were 27 (45%). HER2 positive and negative cases were 28 (32%) and 32 (53%) respectively.

The mean MC count was more in ER and PR positive cases when compared with ER and PR negative cases which was statistically significant (P-value <0.0001). In a study done by Sang J *et al.*,[15] the mean MC density was higher in ER and PR positive cases when compared with ER and PR negative cases. Heidarpour *et al.*[8] has compared the MC percentage with ER and PR status. According to them, MC percentage was more in ER positive cases, and there was no correlation between MC percentage with PR and HER2 status. A study done by Kwon *et al.*[10] showed that the mean MCs are more in ER and PR negative cases. MCs contribute in angiogenesis and help in the invasion of tumor cells. Hence it was concluded that MC count was inversely related to the hormonal status.[10]

In the present study, the mean MC count was higher in HER2 positive cases when compared with HER2 negative, however, the P value was not statistically significant. These observations correlate with Kwon *et al.* study.[10]

Since ER and PR positivity is associated with good prognosis, a greater number of MCs among these cases indicates a better prognosis. Most of the ER and PR positive tumors are positive for MC, therefore, these tumors respond well to hormonal therapy.

CONCLUSION

The maximum number of cases belonged to Grade II according to the modified SBR grading system. The results concluded that MCs in breast cancer were inversely proportional to the grade of tumor. A higher number of MCs was seen in tumors of lower grade suggesting a beneficial role of MCs in breast carcinoma.

An increased number of MCs was observed in ER and PR positive breast cancers since ER and PR positivity is associated with good prognosis and favorable outcome. MCs might act as a new target for the adjuvant treatment of breast cancer through the selective inhibition of angiogenesis and tumor-promoting molecules.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Fakhrjou A, Naghavi-Behzad M, Montazeri V, Karkon-Shayan F, Norouzi-Panahi L, Piri R. The relationship between histologic grades of invasive carcinoma of breast ducts and mast cell infiltration. South Asian J Cancer 2016;5:5-7.
2. Keser SH, Kandemir NO, Ece D, Gecmen GG, Gul AE, Barisik NO, *et al*. Relationship of mast cell density with lymphangiogenesis and prognostic parameters in breast carcinoma. Kaohsiung J Med Sci 2017;33:171-80.
3. Divyarani MN, Harendra Kumar ML, Swaroop Raj BV, Manoranjan UD. Qualitative analysis of mast cells in invasive ductal carcinoma. Int J Curr Res Aca Rev 2014;2:91-6.
4. Della Rovere F, Granata A, Familiari D, D Arrigo G, Mondello B, Basile G, *et al*. Mast cells in invasive ductal breast cancer: Different behavior in high and minimum hormone-receptive cancers. Anticancer Res 2007;27:2465-71.
5. Weller CL, Collington SJ, Williams T, Lamb JR. Mast cells in health and disease. Clin Sci 2011;120:473-84.
6. Rajput AB, Turbin DA, Cheang MC, Voduc DK, Leung S, Gelmon KA, *et al*. Stromal mast cells in invasive breast cancer are a marker of favourable prognosis: A study of 4,444 cases. Breast Cancer Res Treat 2008;107:249-57.
7. TheoharidesTC, Alysandratos KD, Angelidou A, Zhang B. Mast cells and tumor microenvironment. In: The Tumor Microenvironment. New York: Spinger; 2010. p. 353-70.
8. Heidarpour M, Rajabi MA, Rajabi P, Farkhani HS. Mast cells in invasive ductal breast carcinoma. J Res Med Sci 2008;13:255-9.
9. Colditz G, Chia KS. Invasive Breast carcinoma: Introduction and general features. In: Lakhani SR, Ellis IO, Schnitt SJ, Tan PH, Vande Vijver MJ, editors. WHO Classification of Tumours of the Breast. 4th ed. Lyon: International agency for research on cancer; 2012. p. 14-31.
10. Kwon GY, Lee SD, Park ES. Mast cell and macrophage counts and microvessel density in invasive breast carcinoma-comparison analysis with clinico - pathological parameters. Cancer Res Treat 2005;37:103-8.
11. Jagtap SV. Evaluation of CD4+ T-cells and CD8+ T-cells in triple-negative invasive breast cancer. Indian J Pathol Microbiol 2018;61:477-8.
12. Glajcar A, Szpor J, Pacek A, Tyrak KE, Chan F, Streb J, *et al*. The relationship between breast cancer molecular subtypes and mast cell populations in tumor microenvironment. Virchows Arch 2017;470:505-15.
13. Amini R, Aaltonen K, Nevanlinna H, Carvalho R, Salonen L, Heikkilä P, *et al*. Mast cells and eosinophils in invasive breast carcinoma. BMC Cancer 2007;7:165-9.
14. Jana S, Ghosh S, De A, Pal S, Sengupta S, Ghosh T. Quantitative analysis and comparison of mast cells in breast carcinomas and axillary lymph nodes. Clin Cancer Investig J 2017;6:214-8.
15. Sang J, Yi D, Tang X, Zhang Y, Huang T. The associations between mast cell infiltration, clinical features and molecular types of invasive breast cancer. Oncotarget 2016;7:81661-9.